Effect of magnetic hyperthermia on *Pseudomonas fluorescens* planktonic cells and biofilms

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It is known that bacteria can survive disinfection and resist a wide variety of biocidal agents. Nowadays, the synthesis of superparamagnetic nanoparticles and its application in magnetic hyperthermia (MH) is of great interest, with MH being recently reported as a viable alternative to traditional disinfection methods against bacteria. However, fundamental studies comprising a direct comparison of MH effect on planktonic and biofilm cells, or comparing the bactericidal effect of MH with a conventional direct heating (DH) method are scarce. Therefore, this work aimed at evaluating the effect of MH on planktonic cells and biofilms of a food spoilage bacterium – *Pseudomonas fluorescens* - and its performance comparing to DH technique.

The work was performed using a *P. fluorescens* collection strain (ATCC 27663), which planktonic cell suspensions were grown in TSB and adjusted to a final concentration of $\approx 1 \times 10^8$ cells/ml in sterile saline. Biofilms were formed in silicone coupons during 3 days, at room-temperature, with shaking at 120 rpm and also using TSB as culture medium. DH heating assays were performed in a thermoblock, where planktonic cells and biofilms were heated without magnetic nanoparticles (MNPs) added. By its turn, MH assays were performed in specific equipment where an oscillating magnetic field was applied to planktonic cells and biofilms samples in presence of MNPs. In order to evaluate the bactericidal effect of both heating techniques, survival of planktonic and biofilm cells was determined by CFU enumeration. Based on the most relevant results, cell membrane integrity was analysed through CLSM, while SEM was used to evaluate the effects of MH on cells surface and biofilm structure.

Results showed that MH had a higher bactericidal effect than DH, since it promoted a significant cell viability reduction on both kinds of bacterial forms. Moreover, MNPs-based hyperthermia was able to completely eradicate planktonic cells after only ≈ 8 minutes of exposure, corresponding to a final temperature of 55ºC. Nonetheless, biofilms were more tolerant to MH than planktonic cells, possibly due to diffusion limitations along these bacterial communities. CLSM images of planktonic cells and biofilms showed that, although both heating techniques had affected cell membrane integrity, MH has much more harmful when considering the same final temperature. Additionally, SEM pictures revealed that MNPs-based hyperthermia had also affected the bacterial surface and biofilms structure. Altogether, these observations suggest MH's bactericidal effect might be based on a mechanism that targets bacterial cell wall and membrane.

In conclusion, results showed that MH had a better bactericidal performance comparing to a conventional DH, leading to a more efficient reduction of viable planktonic and biofilm cells at lower final temperatures. Even though a higher bactericidal effect was found against planktonic cells over biofilms, the survival of these microbial communities were also more affected by MNPs-based hyperthermia than by conventional heating, and a significant reduction of their viable biomass was accomplished within the temperatures tested. Therefore, this work presents the possibility of using MH out of the biomedical field as a potential method of disinfection in food related environments.

Keywords: magnetite nanoparticles; magnetic hyperthermia; biofilm; planktonic bacteria; *Pseudomonas fluorescens*